PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference T2514-PCT	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/EP 03/09008	International filing date *(day/month/year)* 30.07.2003	Priority date *(day/month/year)* 30.07.2002
International Patent Classification (IPC) or both national classification and IPC A01K67/027		
Applicant TIGENIX N.V. et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 8 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 4 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the opinion
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
26.02.2004	03.12.2004
Name and mailing address of the international preliminary examining authority: European Patent Office - P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Tx: 31 651 epo nl Fax: +31 70 340 - 3016	Authorized Officer Sitch, W Telephone No. +31 70 340-3040

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP 03/09008

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, Pages

1-48 as originally filed

Sequence listings part of the description, Pages

1-13 as originally filed

Claims, Numbers

1-33 received on 10.03.2004 with letter of 08.03.2004

Drawings, Sheets

1/7-7/7 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).

   ☐ the language of publication of the international application (under Rule 48.3(b)).

   ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   ☒ contained in the international application in written form.

   ☒ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority in written form.

   ☐ furnished subsequently to this Authority in computer readable form.

   ☒ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   ☒ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   ☐ the description, pages:

   ☒ the claims, Nos.: 34, 35

   ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

   ☐ the entire international application,

   ☒ claims Nos. 26-29; claims 23-25, 30, 31 with respect to industrial applicability

   because:

   ☒ the said international application, or the said claims Nos. claims 23-25, 30, 31 with respect to industrial applicability relate to the following subject matter which does not require an international preliminary examination (specify):

   see separate sheet

   ☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

   ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

   ☒ no international search report has been established for the said claims Nos. 26-29 due to a finding of lack of unity

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

   ☐ the written form has not been furnished or does not comply with the Standard.

   ☐ the computer readable form has not been furnished or does not comply with the Standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	5, 6, 15-20, 24, 25
	No:	Claims	1-4, 7-14, 21-23, 30-33
Inventive step (IS)	Yes:	Claims	None
	No:	Claims	1-25, 30-33
Industrial applicability (IA)	Yes:	Claims	1-22, 32, 33
	No:	Claims	

2. Citations and explanations

see separate sheet

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. Due to the finding of lack of unity, no search report has been established in respect of original claims 28-31 (present claims 26-29). Following therefrom, in application of the provisions of Rule 66.1(e) PCT, no international preliminary examination is being carried out on the subject matter of said claims.
2. Claims 23-25, 30, 31 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(I) PCT). However, an international search report based on the alleged effects of the products / compositions in question has been established for such claims since their subject matter can readily and in a straightforward manner be understood in terms of these effects. Accordingly, an opinion on novelty and inventive step of the subject matter of these claims is also now given in as far as relating to the alleged effects of the products / compositions in question (see PCT Rule 33.3(b) and 66.1(a)).

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Reference is made to the following documents:

D1: DE BARI ET AL: "Multipotent mesenchymal stem cells from adult human synovial membrane" ARTHRITIS AND RHEUMATISM, vol. 44, no. 8, 2001, pages 1928-1942, XP002266867

D2: DE BARI C ET AL: "Human synovial membrane-derived mesenchymal stem cells for skeletal muscle repair" ONCOLOGY RESEARCH, vol. 12, no. 6-7, 2001, page 284, XP008026328 2001 Millennium International Conference of Molecular and Tumor Biology;Santorini, Greece; September 02-07, 2001 ISSN: 0965-0407

D3: DE BARI C ET AL: "Human mesenchymal stem cells from synovial membrane contribute to skeletal muscle repair in vivo" CLINICAL RHEUMATOLOGY, vol. 20, no. 5, 2001, page 400, XP001184115 5th Belgian Congress on Rheumatology;Hasselt, Belgium; September 27-29, 2001 ISSN: 0770-3198

Attention is drawn in particular to all passages of these documents as indicated on the International Search Report, unless stated otherwise.

1. Preliminary comments

The present application relates broadly to the field of dysfunction and repair of muscle tissue, and seeks to provide suitable material, that is cell populations, for use in restoring damaged or disease muscle, including wherein such restoration is persistent. More specifically, the application relates to the provision of a particular cells population ('synovial membrane derived muscle progenitor cells' or 'SM-MPCs') and their use in therapy.

The position of this examining authority until now and as expressed previously has been that from the prior art, as represented by for example D1, the cell population per se of the present application (the joint tissue derived muscle precursor / progenitor cell population) is known: this is in fact the basis on which the finding of a lack of unity of the present application centres. This position with respect to the cell population of the application would appear to be confirmed by the text on page 21, 2nd paragraph of the present application, referencing D1 (De Bari et al); in addition see page 43, last paragraph, page 30, paragraph 2 of the present application. A comparison of the tissue source (synovial membrane) and method for producing the cells of the present application with the tissue source and method for producing the cells of D1 (page 1929 of D1) would appear to further substantiate the identity of the cells per se of the present application with those of D1. This identity between the two cell populations would appear a justifiable conclusion irrespective of for example the possible identification of further previously unidentified particular markers of the cell population in question in the present application.

In contrast to the above, the applicant has put forward that the cell population as defined in claim 1 is new: the reasons for disputing this and thus for maintenance of the position as set out previously with respect to novelty of the cell population of the present application are given hereinafter.

In claim 1 the cell population in question is defined as being characterised by the 'expression of c-met as a positive marker...' and the 'expression of gdf5/cdmp1 as a negative marker...' The exact meaning of the latter term lacks clarity: the assumption has been made that the intention is to reflect the idea that a cell which expresses gdf5/cdmp1 is not a cell population which is desired to be claimed, or alternatively stated, that the cell population desiring to be claimed does not express this marker. Over the prior art cited, for example D1, such 'negative definition' of a technical feature is considered insufficient to differentiate the cells of the present application from those of the prior art for inter alia the following reasons:

(I) the cells of the prior art and of the present application appear to have been prepared in the same way;
(ii) the apparent absence of a cell marker on a population of cells may arise for various reasons, including for example due to failure to detect the marker with the detection methods used, or due to a transient absence of expression or low levels of expression of the marker;
(iii) in the case that a 'new positive marker' of a known cell population is identified, this does not render the known cell population new. This can be applied analogously in the case of the putative absence of a cell marker, such that (assuming the situation is not as under (ii)) in the case where a cell population is identified amongst a cell population in the prior art which genuinely does not express a particular cell marker, this would seem in itself insufficient evidence that the cell population in question identified as having such 'characteristic' is new. Instead, in order for such to possibly be accepted it would seem reasonable that for example a new (positive) technical feature of the allegedly new cell population be identified, or that for example a new source and/or method of preparation of the allegedly new population be disclosed.

Interestingly, in example 7 on page 43 of the description of this application, is stated that after 8 passages, in about 5% of the cell population with which the application is concerned, the expression of cdmp1 was detected, and in about 20% of the population, c-Met was not detected. Of further interest, at page 46, lines 14, 15 of the description of this application is stated that the 'cells of the present invention [so-called synovial membrane derived muscle progenitor cells, SM-MPCs] were originally described as synovial membrane derived mesenchymal stem cells (SM-MSCs) (De Bari et al [D1])'.

2. Prior Art

D1: Cells per se of the application - see also discussion / justification thereof above. Additional passages of D1 to note: page 1929, 'Materials and Methods', and page 1934.
D2: Further publication of the cells of the application. Stated to act as myogenic progenitor cells, differentiating into skeletal muscle phenotype cells; disclosed as being able to contribute to the repair of chemically injured skeletal muscle (in a nude mouse model). The cells are accessible, expandable, transducable, preservable in liquid nitrogen.
D3: See D2

3. Novelty (Art. 33(2) PCT)

Following from the discussion put forward with respect to the novelty of the cells per se to which the present application relates, in light of the available prior art (D1-D3), the

subject matter of present claims 1-4, 7-14, 21-23, 30-33, at least, lacks novelty. In this regard it should, inter alia, be noted that the therapeutic indication of the cells as disclosed in D2 is considered to fall within the scope of the therapeutic indications recited in second medical use claims 11-14 and 21, 22, and method claims 23, 30, 31. Of note furthermore is that the therapeutic indications of for example claims 21 and 22 ('..for the restoration of Mechano Growth Factor expression ...', '...for the generation of a persistent population of satellite cells') are in all likelihood objectionable as lacking clarity: as mentioned, an objection of lack of novelty of the subject matter of claims 11-14, 21, 22, 23, 30, 31 is in the present instance made.

4. Inventive Step (Art. 33(3) PCT)
Whilst novelty of the subject matter of claims 5, 6, 15-20, 24, 25 may be acknowledgeable over the available prior art, their subject matter would appear derivable by the skilled person as a matter of routine and is thus considered to lack inventive step: the genetic engineering of cells for their use in therapy is a well practised technique (claims 5, 6), the use of the cells of the application in treating further muscular disorders over the disclosure of for example D2 is considered non-inventive (claims 15-18), and the routes of delivery recited in claims 19, 20,24, 25 are routes of delivery well known and practised in the art.

5. For the assessment of the present claims 10-25, 30, 31, 33 (partially) on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

Amendment under Article 19 PCT for international application PCT/EP03/09008

1. A composition comprising a population of mammalian muscle progenitor cells derived from joint tissue, said cells having *in vivo* myogenic properties and providing a persistent pool of satellite cells when introduced into mammals and characterised by the expression of c-met as a positive marker or any marker coexpressed or co-detectable with this positive marker and by the expression of gdf5/cdmp1 as a negative marker or any marker coexpressed or co-detectable with this negative marker.

2. A composition according to claim 1 wherein the cells are derived from synovial membrane.

3. A composition according to claim 1 or 2 wherein the cell population is characterised by the expression of one or more of the synovial fibroblast positive markers CD44 and CD90 and by the absence of the expression of the negative markers flk-1 or any marker coexpressed or co-detectable with these positive and/or negative markers.

4. The composition according to any of claims 1 to 3 further characterised by the expression of CD34 as a positive marker or any marker coexpressed or co-detectable with this positive marker.

5. The composition according to any of claims 1 to 4 wherein the cells are genetically engineered.

6. The composition of claim 5 wherein the genetically engineered cells comprise a promoter operably linked to a nucleotide sequence encoding a protein selected from the group of an angiogenic factor, a peptide growth factor and an anti-angiogenic factor.

7. The composition according to any of claims 1 to 6 wherein the cells are clonal.

8. The composition according to any of claims 1 to 7 wherein the cells are cryopreserved.

9. The composition according to any of claims 1 to 8 wherein the cells are isolated and passaged between 3 and 10 passages.

10. A pharmaceutical composition comprising a composition of muscle progenitor cells according to any of claims 1 to 9 in admixture with at least one pharmaceutically acceptable carrier.

11. Use of a composition according to any of claims 1 to 9 for the manufacture of a medicament for the repair or prevention of a muscle dysfunction.

12. Use according to claim 11, wherein the muscle is skeletal muscle.

13. Use according to claim 11 or 12, wherein the dysfunction is caused by an ischemic event.

14. Use according to claim 11 or 12, wherein the dysfunction is selected from a severe trauma, a diffuse trauma and crush syndrome, disuse atrophy, sarcopenia.

15. Use according to claim 11 or 12, wherein the dysfunction is a muscular dystrophy.

16. Use according to claim 15, wherein the muscular dystrophy is Duchenne Muscular Dystrophy.

17. Use according to claim 11 or 13, wherein the muscle is cardiac muscle.

18. Use according to claim 17 wherein the muscle dysfunction is a cardiovascular disorder selected from myocardial infarct and heart failure.

19. Use according to any of claims 11 to 18 wherein the composition is delivered locally.

20. Use according to any of claims 11 to 18 wherein the composition is delivered systemically.

21. Use of a composition according to any of claims 1 to 9 for the manufacture of a medicament for the restoration of Mechano Growth Factor expression by dystrophic muscle cells.

22. Use of a composition according to any of claims 1 to 9 for the manufacture of a medicament for the generation of a persistent population of satellite cells.

23. A method of regenerating muscle comprising of the step of administrating a composition according to claims 1 to 10 to an individual.

24. The method of claim 23 wherein the composition is injected into the affected muscle.

25. The method of claim 23 wherein the composition is administered into the blood stream.

26. A method of selecting muscle precursor cells comprising the step of simultaneously or subsequently contacting a joint tissue derived cell population with a binding substance for one or more of the positive marker c-Met and/or the negative marker and CDMP1 or any marker coexpressed or co-detectable with this positive or this negative marker.

27. The method according to claim 26 wherein the joint tissue derived cell population is obtained from the synovial membrane.

28. The method of any of claims 25 to 27 wherein the binding substance is an

antibody.

29. The method of claims any of claims 25 to 27 wherein the binding substance is a ligand for a receptor.

30. A method of restoring the capacity of dystrophic muscle cells to express MGF comprising the step of administering a composition according to any of claims 1 to 10 to an individual with dystrophic muscle.

31. A method of providing a persistent reserve population of satellite cells in an individual comprising the step of administering a composition according to any of claims 1 to 10 to an individual.

32. A vehicle for muscle specific delivery of therapeutic agents comprising the composition of claim 5 or 6.

33. The composition according to any of claims 1 to 10, said cells providing after administration to an individual a persistent pool of satellite cells which can contribute to the generation of new myonuclei during muscle regeneration.